STUDIES ON BUFFALO MILK FAT OXIDATION

III.—Isolation and Identification of the Carbonyl Compounds from Oxidized Buffalo Milkfat.

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Mono- and di-cargonyl compounds in oxidized buffalo butteroil and samn averaged 223.4313 umol/10 g and 18.0083 umol/10 g respectively in the former, and 167.7574 umol/10 g, and 13.5551 umol/10 g respectively in the latter. Methyl ketones, saturated aldehydes, 2-enales, and 2,4-dienals are the 4 classes identified from the monocarbonyls fraction. Their maximum light absorption in hexane are 340 mu, 337 mu, 350 mu, and 365 mu wavelengths respectively. The same respective peaks in chloroform are at 362 mu, 358 mu, 369 mu, and 379 mu wavelengths.

The predominant class of the monocarbonyls in oxidized butter oil is the saturated aldehydes, 55%, followed by methylketones; 25%, 2-enals, 12% and 2,4-dienals; 7%. In oxidized samn saturated aldehydes and methyl ketones are equally present; being 40%, while 2-enals, and 2,4- dienals are 11%, and 9% respectively.

According to the mobility on alumina G the following compounds are identified in the following classes:

Methyl ketones : 2-nonacnone, 2-octanone, and acetone.

Saturated aldehydes : n-octadecanal, n-octanal, and n-hexanal.

2-Enals : Octa-2-enal, deca-2-enal, crotonal, and acrolein.

2,4-dienals : Octadeca-2,4-dienal, deca-2,4-dienal, and penta-2, 4-dienal.

The volatile monocarbonyl compounds occurring in oxidized milkfat belong to 3 classes; namely: *a*) the alkanals; staurated aldehydes and methyl ketones; *b*) alk-2-enals, and *c*) alk-2,4-dienals. There are probably traces of other compounds and they may have some influence on the oxidized flavors. Work dealing with the identification of those compounds in buffalo milkfat is almost nill if compared with those made on the cow milkfat. Therefore, the purpose of this study is to isolate and estimate quantitatively the aliphatic non- and a-di-carbonyl compounds from oxidized buffalo mildkfat. The separation of the monocarbonyls fraction to its main classes are carried out, and the resolution of each class to its individual compounds are also tried.

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Experimental and Methods of Analysis

Fresh buffalo milk samples were collected from the herd of the Faculty of Agriculture, Ain-Shams University, and the cream was separated. The fresh cream was washed with distilled water, then churned to butter. A portion of the butter was melted at 55°C, washed with hot distilled water for several times, and the clear butteroil layer was separated and filtered. Another portion of the butter was converted to samn by the boiling off method. Both samples were left to stand in loosly covered flasks in a place in the laboratory where they were exposed to a maximum amount of diffused sunlight and air over a period of about 380 days.

The method of Schwartz, Haller, and Keeney (1963), was followed for the quantitative isolation and determination of the carbonyl compounds. The concentration of the 2, 4-DNP hydrazones of total mono-, and di-carbonyls were estimated by measuring the absorbency of the solution at 340 mu (Schwartz, et al, 1963), and 390 mu (Schwartz, et al, 1962)[1], wavelengths respectively. Using a Beckman Spectraphotometer Model DU with 1 cm glass cells, the readings were converted to umoles using $E = 22,500$ (Schwartz, et al, 1963), and 40,0000 (Schwartz, et al ,1962) respectively.

The 2, 4-DNP hydrazones of the monocarbonyls were fractionated into classes using a magnesia-celite column, as described by Schwartz, et al (1962)[2]. Each class was identified by its characteristic light absorption maxima with chloroform and hexane as solvents. The light absorption maxima used for the 4 classes in chloroform : methyl ketones, 360 mu ; saturated aldehydes, 355 mu ; 2-enals, 370 mu : and 2, 4-dienals 390 mu wavelengths respectively (Mookherjee, and Chang, 1963), while in hexane their respective maxima were 340 mu, 355 mu, and 370 mu wavelengths (Corbin, et al 1960, and Mookherjee, and Chang, 1963). In cases where the MgO was stronger and had a higher I.V. the column was developed by the solvent system of methanol in chloroform described by Mookherjee and Chang (1963). The concentration of each class was estimated quantitatively by measuring the absorbency at the wavelength of light maximum absorption, taking in consideration that E of methylketones, saturated aldehydes, 2-enals, and 2, 4-dienals were 22,500 ;22, 500 ; 27,500 ; and 37,000 respectively (Schwartz et al., 1962).

The components of the different classes were separated by thin-layer chromatography (Nano, 1964) on alumina G, using hexane : etyhl acetate 9.1 v/v, and nitrobenzene : chloroform : hexane, 1 : 2 : 8 v/v, as eluting systems. The yellow spots were scraped and extracted by chloroform, filtered and diluted. The optical density of each individual component was measured at the wavelength of maximum light absorption specific of its class.

Results and Discussion

The carbonyl compounds isolated from oxidized buffalo milkfat in advanced stages were mainly methylketones, saturated aldehydes, 2-enals, 2, 4-dienals, and α-dicarbonyls. Their range of concentrations at different peroxide levels were presented in table 1.

TABLE 1.—AMOUNTS OF P.V., T.M.C., METHYL KETONES, SATURATED ALDEHYDES, 2-ENALS, 2,4-DIENALS, AND α-DICARBONYLS IN OXIDIZED BUTTEROIL AND SAMN

	Percent conc.*	Max.	Min.	Average
Butteroil :				
P.V.	—	886.4000	319.2560	223.3413
Total Monocarbonyls	—	357.2346	130.2701	223.3413
Methyl ketones . . .	25	78.5542	31.5224	56.5691
Saturated aldehydes .	55	207.9310	76.3109	123.6249
2-Enals	12	40.7625	12.8425	27.7152
2, 4-Dienals	9	31.6071	10.9521	19.0453
α-Dicarbonyls	—	23.6624	13.5541	18.0083
Samn :				
P.V.	—	884.4104	804.9578	—
Total Monocarbonyls	—	237.5548	123.5241	167.7574
Methylketones . . .	40	98.4093	45.5231	67.3529
Saturated aldehydes .	40	100.7825	43.9115	67.2952
2-Enals	11	21.2058	13.2887	16.8162
2, 4-Di enals	9	14.4562	10.2155	12.2188
α-Di carbonyls . . .		17.4353	10.7823	13.5551

* Percent concentration of total monocarbonyls.

The distribution of the 4 classes differed in the samples. Generally in oxidized butteroil the saturated aldehydes were the predomenant as they contributed to about 55% of the T.M.C., while the methylketones contributed to about 25% of the T.M.C. The 2-enals, and 2, 4-dienals classes were present in lower percentages, as the 2-enals class were 12% and the 2, 4-dienals class were 9% of the T.M.C. compounds. In oxidized samn, both the saturated aldehydes, and methyl ketones classes were almost present in equal percentages ; namely 40% of each of the T.M.C., while the 2-enals class was only 11%and the 2, 4-dienals class was even less ; being 9% of the TMC.

The higher propertions of the methylketons class found in oxidized samn than in butteroil could be attributed to the effect of heating the butter in the presence of air during the processing of samn. Water which was not completely removed from the samn by this method might also add to the effect.

Several references confirmed the effect of heat on producing a homologous series of n-alkyl members of the methylketones, having odd numbers of carbon in their chains in milkfat. Lawrence (1963), was able to isolate a complete range of methylketones from fresh butteroil and other dairy products. Patton and Tharp (1959), identified a homologous series of methhyl ketones with odd numbers of carbons. They claimed that the fresh unheated milkfat was devoid of such ketones, with the exception of acetone, but contained other carbonyl fractions. Furthermore, Langler and Day (1964), indicated that a large icrease in ketone production occurred when milkfat was heated at temperatures above 100°C in the presence of air and traces of water.

The formation of methyl ketones were attributed by Bolding and Taylor (1962), to the small amounts of B-ketto acids which were present in anbound form in butter, and considered them as the precursors of methyl ketones. By single degradation, they claimed, would yield methyl ketones containing one less carbon atom. Recently Vanderven, et al. (1963), and Parks et al. (1964), confirmed these findings by proving that the methyl ketones precursors would exist in milkfat as B-keto esters.

The presence of water was essential for the formation of methylketones in heated milkfat. B-keto esters required 0.0031% of water for their complete hydrolysis on the liberation of the ketones (Langler and Day 1964 and Schwartz et al. 1965). Therefore, the relatively higher water content of 16% in butter was sufficient to hydrolyze the B-keto acids ester. Consequently the attention must be drawn to the significance of the moisture contents of butteroil and samn that equal to the effect of O_2 in their oxidative deterioration. Butteroil contains about 3% and samn 0.01% water which were enough to form methylketones and cause their flavor deterioration.

The origin of the carbonyl compounds in autoxidized milkfat arises mainly from the autoxidation of the unsaturated acids present in the triglycerides of butteroil. By considering the autoxidation theory of Farmer et al. (1942), and its extension to the α-methylene group outside the non-conjugated 1, 4-pentadienoic system, the formation of all saturated aldehydes, 2-enals, and 2, 4-dienals could be presumed from the auto-oxidation of the unsaturated acids. The mechanism for the formation of 2-alkanones, however, was difficult to postulate. According to Ellis (1950), 2-alkanones could be formed by the addition of water to the double-bond next to the keto-group formed from the hydroperoxide. Another mechanism for the formation of 2-alkanones was the decarboxylation of B-ketoacids (Boldingh and Taylor 1962).

Characterization of each class was facilitated by measuring their light absorption maxima in hexane and chloroform as solvents. These maxima were presented in table 2.

However, these maxima of the classes either in hexane or chloroform were slightly different from these reported by other investigators as Corbin, et al. (1960), Mookherjee at al (1963), and Schwartz et al. (1962). The presence of an anomolous hydrazone in a class such as formaldehyde in the 2-enals class, might lower the light absorption maxima of that class. Therefore,

the methyl ketones, and saturated aldehydes did not show a differnt light absorption maxima from those observed by other investigators either in hexane or chloroform as solvents. The 2-enals class, however, showed slight shifting in the light absorption maxima, while the 2, 4-dienals class showed more pronounced shift especially when measured in chloroform as solvent.

TABLE 2.—Light absorption maxima of methyl ketones, saturated aldehydes, 2-enals, and 2, 4-dienals classes in hexane and chloroform.

Classes	In hexane mu	In chloroform mu
Methyl ketones	340	362
Saturated aldehydes	337	358
2-Enals	350	369
2, 4-Dienals	365	379

Methyl ketones, saturated aldehydes, and 2, 4-dienals classes of both oxidized butteroil and samn each contained 3 individual compounds, while the 2-enals class contained 4 components, according to their mobility, starting with component A as the least mobile component, as shown in Figs. 1,2, and table 3.

The individual components were also identified from their light maximum absorption, when compared with those in the literature. Refering to the results of Schwartz et al. (1961[1],1962[2]), the 3 components. of the methyl ketones class having light absorption, maxima at 362 mu wavelength were identified as 2-nonadecanone for component A, 2-octanone or 2-nonanone for component B, and acetone for component C. Confirming the previous deduction, Jones et al. (1956), presented a light absorption peaks at 364 mu wavelength for acentone and 365 mu wavelength for 2-nonadecanone, and 2-nonanone. The components of the saturated aldehydes class having peaks at 358 mu wavelength were identified as n-octadecanal for component A, n-octanal for component B, and butanal, hexanal or acetaldehyde for component C, according to Schwartz et al. (1962[1]). On the other hand, Jones et al. (1956), gave a light absorption peak at 354 mu wavelength for acetaldehyde, and 359 mu wavelength for n-butanal hexanal. Therefore, component C might probably identified as n-butanal or n-hexanal rather acetaldehyde. The components of the 2-enals class having peaks at 369 mu wevelength, were most probably octadeca-2-enal for component A, deca-2-enal for component B, crotonal for component C, and acrolein for component D, with reference to the results of Schwertz et al. (1962[1], 1962[2]). Furthermore,

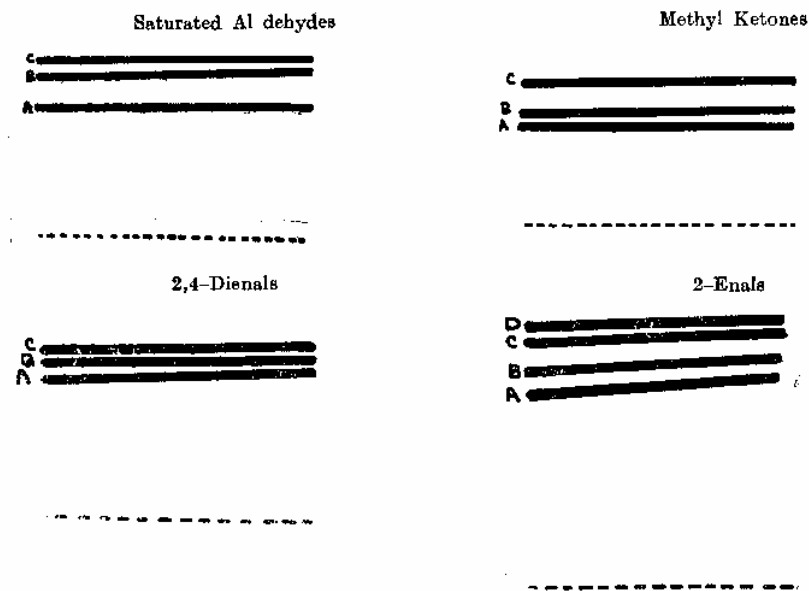Saturated Al dehydes   Methyl Ketones

FIG. 1.—Thin-Layer Chromatograms of the Methyl ketones, Saturated aldehydes, 2-Enals, and 2,4-Dienals of Butteroil, Using Hexane : Ethyl Acetate, 9 : 1 V/V, on Alumina G.

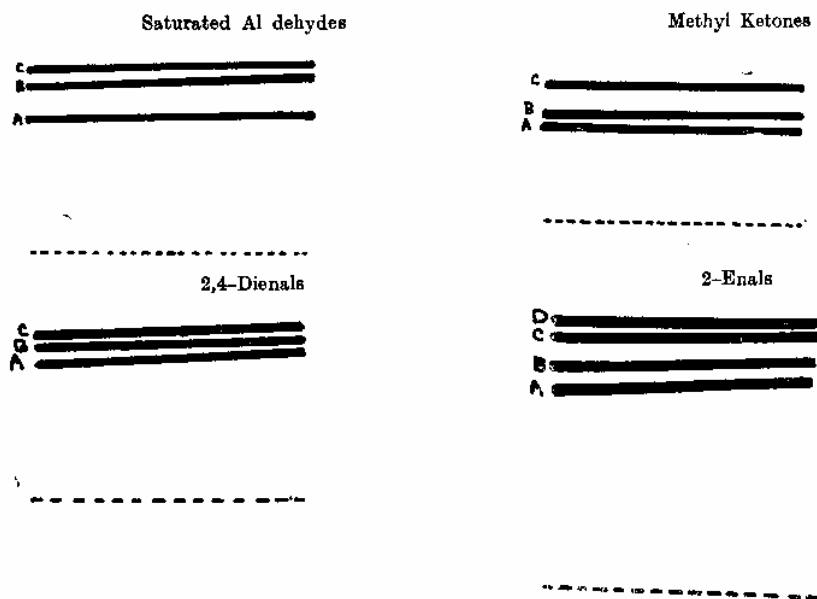Saturated Al dehydes   Methyl Ketones

FIG. 2.—Thin-Layer Chromatograms of The Methyl ketones, Saturated aldehydes, 2-Enals, and 2,4-Dienals of Butteroil, Using Hexane : Ethyl Acetate, 9 : 1 V/V, on Alumina G.

Jones et al. (1956), gave a light absorption peaks at 368 mu, and 373 mu wavelengths for acrolein and crotonal respectively. The 3 components of the 2,4-dienals, which peaks at 379 mu wavelength could be identified according to the results of Schwatz et al. (1962[1], 1962[2]), to octadeca, 2,4-dienal for compenent A, deca-2,4-dienal for component B, and penta 2,4-dienals for compenent C.

TABLE 3.—AMOUNT OF THE 2,4-DNP HYDRAZONES OF METHYL KETONES, SATURATED ALDEHYDES, 2-ENALS, AND 2,4-DIENALS CLASS IN OXIDIZED BUTTEROIL AND SAMN.

	Butteroil			Samn		
	Max.	Min.	Average	Max.	Min.	Average
Methylketones :						
A (2-nonannone)	37.6037	10.2840	17.0851	30.2314	14.8231	22.5819
B (2-octanone)	52.2114	15.3362	31.0972	46.0074	20.0932	28.6521
C (acetone) . . .	10.9003	4.3879	8.2692	23.8921	9.8941	15.5643
Saturated aldehydes :						
A (n-octa-decanal)	121.9481	37.5723	65.1493	45.0312	19.0021	29.8281
B (n-octanal) . .	64.3261	21.4187	36.1436	36.7132	13.7741	22.8428
C (n-hexanal) . .	24.4865	15.7426	20.8781	20.2245	10.1142	13.5131
2-Enals :						
A (octa-2-enal) .	12.9248	4.3362	8.3987	6.7145	3.5523	4.9658
B (deca-2-enal). .	10.2314	4.1246	7.1392	5.7431	2.7483	4.5394
C (crotonal) . .	9.4781	3.4462	6.4572	4.9248	2.5094	4.3114
D (acrolein) . .	8.7236	2.3314	5.6120	4.3114	2.3364	3.2169
2,4-Dienals :						
A (octadeca-2,4-dienal)	11.8953	4.2234	7.1209	4.7421	3.0246	4.0005
B (deca-2,4-dienal)	9.2331	3.5721	6.1057	4.3871	3.3365	3.8823
C (Penta-2,4-dienal)	8.7432	2.4268	4.7968	3.8742	2.3374	3.0336

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دراسة على أكسدة دهن اللبن الجاموسى

٣ ــ فصل وتمييز المركبات انكربونيلية الناتجة من اكسدة دهن اللبن الجاموسى

نوال سيد أحمد ــ ابراهيم الدسوقى رفعت ــ عبد الحميد الحوفى

أمين محمد السكرى

الملخص

كان متوسط تركيز المونو ــ كربونيلات داى ــ كربونيلات فى دهن الزبد المسيل المتأكسد ٣١٢ر٤٢٢٣ مليمول / ١٠ جم ، ٨٣ر١٨٠٠ مليمول / ١٠ جم على التوالى بينما كان تركيزهما المناظر فى حالة السمن المتأكسد ٧٥٧٤ر١٦٧ مليمول/١٠ جم ، ٥٥٥١ر١٣ مليمول/١٠ جم يحتوى المونو ــ كربونيلات فى كل من دهن الزبد المسيل والسمن الجاموسى المتأكسد على مجموعات . المثيل كينونات ، الألدهيدات المشبعة ٢ ــ اينال ، ٢ر٤ ــ داى اينال وكان اقصى امتصاص للضوء عند استعمال الهكسان كمذيب على موجة ضوئية طولها ٣٤٠ ، ٣٣٧ ، ٣٥٠ ، ٣٦٥ للميكرون بالنسبة للأربع مجموعات على التوالى . أما عند استعمال الكلوروفورم كمذيب كان طول الموجة ٣٦٢ ، ٣٦٨ ، ٣٦٩ ، ٣٧٩ مليمكرون على التوالى وكانت مجموعة الالدهيدات المشبعة السائدة فى حالة دهن الزبد المسيل المتأكسد وصلت نسبتها الى ٥٥٪ ويليها مجموعة الميثيل كينونات بنسبة قدرها ٢٥٪ ثم ٢ ــ اينال بنسبة ١٢٪ واخيرا مجموعة ١ر٤ ــ داى اينال بنسبة ٩٪ . اما فى حالة السمن المتأكسد فكانت نسبة مجموعتى الميثيل كينونات والدهيدات المشبعة متماثلة تقريبا بمتوسط قدره ٤٠٪ لكل منهما بينما كانت نسبة مجموعة ٢ ــ اينال ١١٪ ثم مجموعة ٢ر٤ ــ داى اينال بنسبة ٩٪ . بالرجوع الى سرعة الجريان على سطح اكسيد الالومنيوم امكن فصل هذه المركبات من المجموعات المختلفة .

الميثيل كينونات : ٢ ــ نوناديكانون ، ٢ ــ اكتانون ، الاسيتون .

الألدهيدات المشبعة : اكتاديكانال ، اكتانال ، هكسانال .

٢ ــ اينال : اكتاديكا ــ ٢ ــ اينال ، ديكا ــ ٢ اينال ، الكرتونال ، والاكرولين .

٢ر٤ ــ داى اينال : اكتاد ــ ٢ر٤ ــ داى اينال ، ديكا ٢ر٤ ــ داى اينال بتنا ــ ٢ر٤ ــ داى اينال .